

# SEROLOGICAL WASSERMANN "PROBLEM" CASES\*

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The subject of Wassermann "Problem" cases is of interest not only to the serologist but to the physician or clinician, upon whom devolves the responsibility for diagnosis. In the past there has been too little critical judgment by clinicians in assessing the results of the Wassermann reaction, and many practitioners may still regard a positive Wassermann reaction as in itself a diagnosis of syphilis.

In recent years there has been an enormous increase in the practice of serum testing for syphilis. In general hospitals a routine Wassermann test is now carried out on all donors, and often on recipients for blood transfusion. A blood test is now frequently asked for before marriage. At all big venereal disease clinics and ante-natal clinics a routine blood test is done on every case. It is therefore of great importance to the clinician to realize the limitations of tests for syphilis, for it is now known that these serum tests may give occasional false positive results, and there is no doubt that in consequence a certain number of patients in the past have been subjected to anti-syphilitic treatment on account of a positive Wassermann report which further investigation would have proved false.

In approaching this subject it is not my intention to delve into an abstruse scientific discussion regarding the biological causes for false positive Wassermann reactions, but rather in a practical manner to mention the views held and to emphasize the limitations and pitfalls as a whole, and to stimulate discussion on the interpretations that should be placed on a positive Wassermann reaction which is not supported by the clinical data. In 1943 in this *Journal* Harrison and Osmond

called attention to the limitations of serum tests for syphilis. These authors particularly discuss the causes, prevention, and detection of false positive reactions and the prevention of a false diagnosis of syphilis when a positive Wassermann reaction is not supported by the clinical evidence. This contribution at the time was badly needed in order to emphasize the limitations of the Wassermann test.

False positives are generally classified as either technical or biological.

## Technical Causes

Since the discovery of the Wassermann test by Wassermann, Neisser, and Bruck in 1906, many variations in the technique have been devised by different workers, and consequently indifferent results are often obtained. In this country we are fortunate in having a standard Wassermann technique, and credit must be given to Colonel Harrison for his work on standardization. The No. 1, or Harrison, method reported in the Medical Research Committee Special Report Series No. 14 in 1918, and later described in detail by the late Dr. Wyler (1929) in the Medical Research Council's Report Series No. 129, and now commonly known as the Harrison-Wyler method, has been subjected to very thorough comparison tests against other methods, not only in this country but abroad, and its reliability has been conclusively proved. When it is competently carried out and the details followed, and carefully standardized reagents used, it is one of the most reliable pathological tests we have.

The Medical Research Committee (1918) on the Standardization of Pathological Methods stated: "In the opinion of the Committee there is no process of bio-chemical diagnosis

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that gives more trustworthy information or is liable to a smaller margin of error than the Wassermann test when it is performed with completeness and with proper skill and care." Harrison and Osmond (1943) say: "This statement is probably as true to-day as it was when written a quarter of a century ago, but it is equally true that no group of tests has given rise through unskilful performance and through inadequate appreciation of their limitations to more unhappiness than have the serum tests for syphilis."

The performance of the Wassermann test requires skill and concentration. Any slackness may give rise to some technical error. Suppose, for example, there is a batch of seventy blood sera to be tested. This entails the drawing off in pipettes of sera from each numbered tube into another series of numbered tubes for one in five dilution with saline for the purpose of inactivation at 55° C. After inactivation, each diluted serum has to be placed in further correspondingly numbered tubes for the actual test. All this requires patience and care; otherwise a mistake may easily be made. Many anomalous results may have been reported in the past by unskilled technicians, not because the Wassermann test was at fault, but because the operator was at fault. In a well-run laboratory, and when a competent technician is performing the test, a mistake is seldom made.

Recently a patient was sent to the Endell Street Venereal Disease Clinic because his serum had been tested by a laboratory and reported as giving a double-plus Wassermann reaction. At the Endell Street Clinic the Wassermann and Kahn tests were twice found negative. At the same time a careful clinical examination revealed no history or clinical evidence of syphilis. As a further check the Tests were once more repeated, this time at two separate clinics, and the reports from both were negative. It was agreed that the first reported (+ +) Wassermann must have been a technical error and that the patient was not suffering from syphilis.

To prevent such mistakes, whenever a positive or doubtful result is reported which is not supported by clinical findings, a further specimen should be sent for verification. The Kahn test is also useful as a check on the Wassermann. In this way the risk of making a technical error is diminished. If the pathologist feels that there is still doubt in the mind of the clinician, he should have no hesitation

in asking for a blood specimen to be split and sent to another laboratory for comparison and verification.

There is also the possibility of a technical error in numbering or labelling specimens. Or occasionally a blood specimen is badly hæmolized, so that on inactivation at 55° C. it becomes a solid chocolate-like mass. Such a serum is, of course, unsatisfactory for testing, and if such instances occur the method of taking blood specimens at the clinic should be overhauled. Another possible source of error (mentioned by Orsós, 1936) is an admixture of salvarsan (commonly from using the syringe with which an injection has just been made). Such a contamination even in high dilution readily leads to false positive results. Therefore separate syringes should be kept for taking specimens and not used for anything else. A further frequent technical mistake is bacterial contamination of a specimen. Sterile tubes and sterile syringes should be used, and the specimen, especially in hot weather, kept in the refrigerator.

#### Lability of Biological False Positive Reactions

There is now almost conclusive evidence to indicate that in some apparently normal individuals there may be a peculiar lability of the blood serum, either permanently or under the influence of some intercurrent disease, which may cause it to give a false positive reaction to Wassermann and other syphilis tests. This peculiar lability has been known for some time, and there is now an accumulating literature on the occurrence of such false positive reactions in certain diseases. It has been long known that animal sera, particularly rabbits, can give positive Wassermann reactions and flocculation tests.

Albrecht (1942) observed that normal rabbits gave 1·21 per cent. positive reactions with the Chediak dried-blood modification of the Meinicke test. He further observed that the reactions given by such rabbits were un-influenced by heat or cold, overfeeding or starvation, or by any intercurrent infection such as catarrh or gastritis.

Sachs (1942) published a paper on some aspects of the serology of syphilis, and discussed the question whether antibodies alone are responsible for complement fixation and

flocculation following the interaction between serum and tissue extracts. He states that although opinions in this respect may differ, it is unquestionable that another mechanism of serological reactivity exists, apart from that caused by antibody action.

According to Sachs "The reagents (tissue extracts, etc.) used for the serological syphilis tests are able also to react with the proteins of labile sera. The results of such an interaction are just the same as in true antibody reactions, complement fixation, or flocculation. The difference is due only to the fact that in these circumstances the extract components react immediately with the serum globulins while the specific reaction between antibody and antigen (or hapten) is secondarily followed by the same alterations inducing complement fixation or flocculation. This consideration makes non-specific reactions in the sero-diagnosis of syphilis understandable. It must be realized that the extracts used as "antigens" are not at all pure reagents. The specific component responsible for the antibody reaction is only a small share (chemically not sufficiently recognizable) which adheres to a big portion of ballast material originating from the tissues. This ballast material and its colloidal structure is of great importance because it may act like antigens—so to speak as 'pseudo-antigens'—but only because of its colloidal behaviour. The result is a non-specific reaction which will occur the more easily the more labile the serum globulins."

Another explanation put forward assumes that non-specific reactions may be caused by antibodies related to those developing in syphilis or caused by symptomless infections. Sachs states that, although antibodies identical with those characteristic of syphilis may be produced in some other infectious diseases such as yaws, malaria, leprosy, and subacute bacterial endocarditis, he does not believe in the theory which endeavours to explain non-specific reactions in general by the same mechanism as that responsible for the Wassermann reaction.

Sachs points out "that non-specific reactions are more frequent and stronger when unheated serum is used instead of heated serum. Heating the serum, first recommended for the intensification of the Wassermann reaction on account of the destruction of the complement activity, just weakens the reactivity and increases at the same time the specificity. An unheated serum of a pregnant non-syphilitic woman, e.g., may give a stronger complement fixation than a heated syphilitic serum, while the reactivity disappears completely after heating. On this account heating

the sera at 55° C. for half an hour is one of the most important measures to warrant the reliability of nearly all serological syphilis tests. In flocculation tests, moreover, the sensitivity is increased in such a way. Only when a more concentrated sodium chloride solution is used as medium, as in Meinicke's clarification test, may unheated serum be used. The higher salt concentration prevents the reactivity of labile globulins and acts, therefore, in the same manner as heating which causes their stabilization."

Sachs therefore claims that it may be justifiable to assume a general biological mechanism for the lability reactions. Actually it is possible to obtain with nearly all sera a positive Wassermann reaction, either if the sera are unheated or if the salt concentration is diminished. Both are understandable—the unheated serum yielding a sufficient lability, and the diminution of the salt content facilitating the reactivity of the labile serum globulins. Sachs, in certain experiments with Dr. Havelock Nelson, observed that "in certain circumstances on using hypersensitive extract dilutions flocculation may be obtained with all sera at room temperature, but that after a subsequent stay in the incubator only flocculations produced by syphilitic serum remain while the others dissolve. This shows that a degree of non-specific reactivity may be present in every serum."

Thus, we may assume that in the Wassermann test there are two types of reactivity possible, viz. (1) antibody reactions (syphilitic type); (2) lability reactions (general biological type) responsible for false positive reactions.

Sachs states that "complement fixation and flocculation are the consequence of the same alteration of globulins which may be caused either specifically and indirectly by antibody action or non-specifically and directly by serum lability. Although the flocculation tests are usually more sensitive than the Wassermann reaction, occasionally the opposite result may be obtained. The reason for this is that complement fixation occurs best when the antigen-antibody complexes are in the stage of development and sometimes because of the dependence on optimum proportions just in the circumstances that do not induce a visible flocculation. On the other hand, complement fixation may be inhibited by the non-specific components of the serum. Such an influence may cause a negative Wassermann reaction while flocculation is positive, although complement fixation is more sensitive in principle. Moreover,

under suitable conditions, the Wassermann reaction is most specific. Because of these peculiarities, both complement fixation and flocculation must be used if the true evaluation of a suspected syphilitic serum is desired."

Kahn (1942 ; 1943) made a special study of the differential characteristics of non-specific and specific serological reactions. He observed that a non-specific reaction is weakened at 37° C. and strengthened at 0° C. ; conversely specific reactions are strengthened at 37° C. and weakened at 0° C. He also found that tissue extract antigens of excessive sensitivity can be prepared which will give about 40 per cent. positive serological reactions in non-syphilitic persons. If the tests with excessively sensitive antigens are performed at cold temperatures the non-specific sensitivity can be raised to about 80 per cent. If the tests with excessively sensitive antigens are performed at cold temperatures with unheated sera instead of with sera heated for 30 minutes at 56° C., the non-specific sensitivity reaches about 98 per cent. Certain lower animals give 100 per cent. of positive reactions when the tests are performed under the same conditions.

The above findings suggest that there exists what Kahn calls a "universal" serological (non-syphilitic reaction) given by human beings and animals, but that it is best observed under conditions of low temperature.

A further interesting observation made by Kahn is the possibility of biological false positive reaction in the presence of syphilis, e.g. a certain person has a tendency to give false positives, let us say during a cold. In due time he becomes infected with syphilis and begins to show specific positive reactions. Then, when he contracts a cold, he is likely to give at the same time both specific and non-specific reactions. Should he later become specifically serum-negative following therapy, he will give the non-specific reaction only when he has a cold. Similarly, certain neuro-syphilitic patients treated with malaria give positive specific serological reactions before malaria therapy, both specific and non-specific reactions soon after malaria, and in due time only specific serological reactions.

Kahn has, in consequence, developed a new Kahn verification test which can be applied to those difficult "problem" cases when a positive Wassermann reaction is reported in the absence of clinical evidence of syphilis.

If the Kahn verification test indicates in such a case a non-luetic type of reaction, one might be justified in assuming a biological false reaction and that the case is probably non-syphilitic.

#### Diseases in which Pseudo-positive Wassermann Reactions have been Recorded

The following are diseases in which pseudo-positive reactions have been recorded :

- yaws
- malaria
- trypanosomiasis
- kala-azar
- African tick fever
- pellagra
- beri-beri
- relapsing fever
- rat-bite fever
- leprosy
- typhus fever
- hepatic distomiasis
- tropical ulcer
- chronic diseases of liver
- scarlet fever
- measles
- diabetes mellitus
- tuberculosis
- lupus erythematosus
- malignant tumours
- sclerodermia
- glandular fever
- (infective mononucleosis)
- German measles
- atypical pneumonia and transient bronchopneumonia
- Vincent's angina
- various subacute inflammations of unknown origin
- staphylococcal septicaemia
- eclampsia
- lymphatic leukaemia

This list of diseases is formidable. The literature on the serological tests for syphilis is enormous—Eagle (1937) in his book on the laboratory diagnosis of syphilis gives over 1,000 references alone. I shall, therefore, confine my remarks to a few outstanding features that may be of interest.

In yaws a positive Wassermann reaction occurs after three or four weeks in over 80 per cent. of active cases. Butler (1936) insists that yaws is syphilis modified by race, climate, immunity, extragenital infection in childhood, and absence of specific immunity. Davis (1944) reviews the findings of the American Committee on Medical Research of the Office of Scientific Research and Development. According to their findings false positive

serological tests are common (more than 10 per cent. of cases) in leprosy, malaria in the acute stages, infectious mononucleosis, vaccination against smallpox, rat-bite fever due to spirillum minus, relapsing fever, lupus erythematosus, and possibly certain types of atypical pneumonia. They state that there is no reliable evidence that the serological tests are significantly affected by pregnancy, menstruation, scarlet fever, jaundice (other than infectious), subacute bacterial endocarditis, tuberculosis, or hypoproteinæmia; and that there is inadequate data available regarding the incidence in measles, mumps, infectious hepatitis, lymphopathia venereum, chancroid, and many other diseases. It is mentioned that transient false positive reactions may occur in apparently normal persons without recent illness. Even persistently positive reactions may occur in non-syphilitic patients. Since a large proportion of sero-positive patients have no syphilitic lesions at necropsy, it is entirely possible that many sero-positive persons without a history or signs of the disease have been mistakenly diagnosed and treated for latent syphilis. The incidence of transient false positive tests following acute infections depends largely on the frequency of testing during the acute and convalescent stages. Although post-infectious or post-vaccinal positive reactions occasionally last as long as three months, most become negative within a few days or weeks. The Committee state that, since it is customary to perform serological tests on hospital patients only on admission, at which time acute infections have not fully developed their antibodies, it is likely that the ability of many common infections to lead to false positive serological tests is grossly underestimated.

Gelli (1931), after a bibliographic review of aspecific Wassermann reactions, discusses the peculiar observation of a four-plus Wassermann sero-diagnosis in a girl aged 11 with hepatic distomiasis, in whom there were no signs pointing to syphilis and in whom the reaction soon became negative when, following intensive treatment, the daily search for ova of *Distoma* proved negative.

Newham (1927) describes a case in which the serum of a patient suffering from liver abscess gave a positive Wassermann reaction on the day after the abscess was evacuated as well as a week and two weeks later, but which was

completely negative to tests carried out three and four weeks after the operation. Syphilis could be excluded in this case, and the author believes that the reaction was due to the tropical abscess. He mentions also a case in which a tube containing a serum which was used as a negative control was on one occasion very slow in clearing. On enquiry he found that the patient from whom the serum had been obtained had an hour previously received a dose of carbon tetrachloride for ankylostomiasis.

Verdozzi and Urbani (1916) give a tabular and detailed account of twenty-six patients with chronic hepatic infections unassociated with syphilis. A positive reaction was obtained in twenty out of the twenty-six patients. Nine of the twenty-six were suffering from primary or secondary new growths of the liver, and eight of these gave a positive reaction; in seven the reaction was strongly positive.

Shinobu Matsumura (1934) tested the Wassermann reaction in 397 cases of malignant tumour, 336 carcinomas, and 61 sarcomas of different parts of the body. The reaction was positive in about a seventh of the carcinomas and about a fifth of the sarcomas. No clinical evidence of syphilis could be found in about half the cases of both carcinoma and sarcoma, and in many of them even no evidence at necropsy. They conclude that a positive Wassermann reaction does not necessarily indicate syphilis either in sarcoma or carcinoma.

Harrison and Osmond (1943) mention a number of references in the literature regarding the occurrence of false positives in glandular fever and after recent vaccination. They also refer to cases in the literature of transient bronchopneumonia, with very little constitutional disturbance, which may cause a positive reaction persisting for two months or more.

Kaufman (1941) found a false positive Wassermann reaction in glandular fever which persisted for as long as two months.

Chargin and Rein (1941) recorded a list of 253 "problem" cases. In some of these cases, in which the blood had sometimes been positive and sometimes negative, no one had been able to say whether the person concerned had ever had syphilis. Cumming and others (1935) give the percentage of false positive reactions obtained by thirteen pathologists, each employing his own method. These

varied from 42 per cent. to 72 per cent. in leprosy ; 0 per cent. to 7.7 per cent. in tuberculosis ; 0 per cent. to 9.7 per cent. in malignant disease ; 0 per cent. to 8.9 per cent. in fever other than malaria, natural or induced ; 8.6 per cent. to 20.6 per cent. in malaria ; 0 per cent. to 3.9 per cent. in jaundice ; 0 per cent. to 3.8 per cent. in pregnancy.

Boas and Neergaard (1934) state that with the technique employed at the State Serum Institute (Copenhagen) a false positive Wassermann reaction in febrile pulmonary disorders seems to be rare (less than 0.33 per cent.), yet in view of the results of other investigations they advise a certain degree of caution in judging the serological test in the presence of an acute highly febrile lung disorder.

Storp (1924) reports a positive Wassermann reaction during the course of a staphylococcal septicæmia in a child aged three years whose parents were free from syphilis. At the end of fifteen days the temperature fell to normal and the Wassermann became negative. In view of this case the author set himself to investigate whether an analogous phenomenon might not be observed in other septicæmias. He studied generalized streptococcal and pneumococcal infections, but found the Wassermann reaction to remain negative.

#### Other Factors Giving Rise to False Positive Results

Other factors which may cause pseudo-positive reactions are : vaccination against smallpox ; administration of animal sera, diphtheria antitoxin, etc. ; general anæsthesia ; pregnancy ; administration of certain drugs ; high protein diet ; serum of persons " in articulo mortis," and corpses ; transient false positives in apparently normal persons with recent illness.

*Diphtheria.*—Frei (1929) confirmed the statement of Hentschell and Szegö that the sera of non-syphilitic patients can give rise to false positive syphilitic reactions after injections of diphtheria antitoxin.

Boas and Tölböll (1932) tested by the Wassermann and Kahn methods the sera of a hundred patients before and after injections of anti-diphtheria antitoxin. All were negative before, and ninety-nine were negative after the injection of anti-diphtheria serum. In the case that was positive the patient received 80,000 units intra-

venously and 140,000 intramuscularly. The tests were carried out by separate assistants and became positive six days later. Eight days later the serum became completely negative.

Stern (1932) states that there have been several reports of positive Wassermann reactions after injection of diphtheria serum. The accuracy of the observations cannot be doubted, but the author advises caution in their interpretation. He observed cases in which the diagnosis was diphtheria but in which the bacteriological examinations did not confirm the clinical impression. No antitoxin was administered in these cases, but in some of them a transient positive reaction of the serum for syphilis was obtained. Positive reactions for syphilis were also observed after Vincent's angina and infections with spirochætes of balanitis. In other words, positive serological reactions for syphilis may occur after infections of the throat without the administration of diphtheria serum, but these usually disappear spontaneously. However, they may persist for longer periods if the infectious foci persist.

*Anæsthetics.*—In a few cases the sera of patients under general anæsthesia have been stated to show a positive reaction. Green (1923) investigated the effect of chloroform and ether upon the Wassermann reaction and the syphilis reaction of Dreyer and Ward. The anæsthetics had no effect upon either reaction. It is generally believed that anæsthesia increases the anti-complementary properties of the blood, special care being required in the performance of the Wassermann test to avoid false positive reports. The Kahn test, not being influenced by anti-complementary properties of serum, is still less affected by anæsthesia. There is no conclusive experimental evidence for the assumption that either test gives false positive reactions during anæsthesia. Nevertheless, as a factor of safety, no serological reaction obtained during anæsthesia should be depended on, particularly if not supported by clinical observations. At least forty-eight hours should elapse between a general anæsthetic and the drawing of blood for a serological test.

*Lead Poisoning.*—Dreyer (1911) obtained positive Wassermann reactions in certain cases of lead poisoning. In thirty-five cases examined, four showed a positive reaction although the men had never been syphilitic. Schnitter (1911) investigated sixteen workmen presenting symptoms of lead poisoning : four showed a positive reaction ; one of these men had suffered from syphilis three years before, although no symptoms of the disease existed at the time of examination. The men giving a positive reaction had been engaged in the lead work for periods varying from eighteen to forty weeks, and the others for periods of from two days to seven weeks ; it is, therefore, probable that

the positive reaction in lead poisoning depends on the degree and the duration of the poisoning.

*Intravenous Injections of Colloidal Silver.*—Since the Wassermann reaction is essentially a colloidal phenomenon, Picado (1917) felt it might be interesting to ascertain the effect of the intravenous injections of colloidal substances upon the reaction. It was found that colloidal silver influences the Wassermann reaction either by reactivation or by inhibition. It may even bring about a positive reaction in normal individuals. These facts must be taken into account in making examinations of persons recently treated with colloidal substances.

*Digitalis.*—In order to verify Bauer's statement that administration of digitalis may produce non-specific positive reactions, Nicoletti (1929) carried out Wassermann tests in persons treated with digitalis preparations. He administered digitalis to forty-five Wassermann-negative persons, in none of whom was a positive Wassermann produced.

*Quinine.*—It has been stated that the administration of quinine may produce a transient positive Wassermann reaction, but this has not been confirmed.

*Iodoform Solution.*—Gjorgjevitch and Pievatchevitch (1927) injected twenty rabbits intravenously with an iodoform solution. After ten or twelve injections the Wassermann reaction became positive in the blood of the animals and remained so for a few days. Subcutaneous injections had no effect. Eleven patients with a negative Wassermann reaction were injected intravenously with increasing doses (from 0.05 mg. to 0.25 mg.) of iodoform in 3 or 4 c.cm. of water. The Wassermann reaction became positive after ten or twelve injections, totalling 1.5 g. of iodoform. A positive Wassermann reaction may, therefore, occur in patients with soft chancres treated for long periods by iodoform.

Green (1923) investigated the effect of various drugs—for example morphine, caffeine, amyl nitrate, epinephrine, pituitary extract—and of fat digestion and fever upon the Wassermann reaction and the reaction of Dreyer and Ward. They had no effect upon either reaction. He states that the presence of considerable fat in the blood causes the serum to become anti-complementary, a property it loses upon the mechanical removal of the fat.

*Corpses.*—Bertolozzi (1934) carried out serological syphilis tests on fifty corpses. A positive reaction was occasionally observed in the serum of persons *in articulo mortis* and in that of a large percentage of corpses.

*Protein Diet.*—Barnes and others (1943) record a case of strongly positive Kahn and Kolmer Wassermann reactions in a young student who denied venereal exposure. This student in the

course of work in a meat market had developed a habit of eating small pieces of raw meat picked up from the chopping block. On the possibility of a high protein diet being the cause of a false positive he was told to stop the practice. In a few weeks his serum became negative. These authors adopted the practice of putting persons whose sera gave contradictory results on a milk-free, meat-free diet for three days, and they give examples of reversals of serum reactions after the institution of this regime; on the other hand they never succeeded in making the serum again positive by restoring the high-protein diet.

*Sulphur dioxide.*—Marsh (1945) recorded an instance of a "control" negative serum becoming positive after keeping a fortnight in a refrigerator. After a lengthy investigation he found that further specimens of sera originally negative gave positive Wassermann reactions when kept for a week or longer in a certain refrigerator. The refrigerator in question had been in constant use for over four years. No mechanical defect nor leakage of gas could be detected by the refrigerating engineers. The refrigerant was sulphur dioxide, and he suggests as a cause a leakage of gas too slight to be detected by ordinary engineering methods.

### Concluding Remarks

It may be asked whether there are any statistics in this country regarding the incidence of non-specific reactions with the Harrison-Wyler technique. I personally know of none, and should like to hear, during the discussion to follow, if there is any information on the subject.

In America, Stokes and others (1946), sponsored by the Committee on Medical Research and the Venereal Diseases Subcommittee of the National Research Council, give statistics over a nine-months' period regarding the occurrence of non-specific positive (so called "biological false positive") reactions for syphilis among Red Cross blood donors. In all 210,261 blood specimens were tested. Out of this number 489 (0.23 per cent.) gave positive results. Of 79 unselected donors with positive serological tests submitted to further extended clinical and special serological studies, only 40.5 per cent. were finally adjudged by a reviewing board to have syphilis. The remainder, 59.5 per cent., were adjudged to have actual or probable false or non-specific reactions. In the process of evaluation a diagnosis of syphilis was reached in three months in 69.2 per cent.; in non-specific positive cases 78 per

cent. required more than three months for a decision.

We now come to the question how best to guard against a false diagnosis of syphilis when an unexpected positive result is reported and where there are no clinical signs or history of syphilis. First, any technical laboratory error must be excluded by repeating the Wassermann tests in conjunction with a Kahn test, and submitting a specimen to a central laboratory or other reliable laboratory as a check.

Harrison and Osmond (1943) recommend that any doubtful reaction obtained by the Harrison-Wyler modification should be subjected to the Richardson modification, which is said to weaken non-specific reactions and strengthen specific ones. Further, such sera should also be subjected to the Kahn Verification test with a view to determining whether the reaction indicates a specific or a non-specific type of reaction.

While these tests may help, it must be recognized that we have no certain serological verification test at present. Let us hope that in the near future some specific physiochemical test for non-specificity will be devised.

### Summary

The serological warnings of non-specificity may be summed up as follows:

1. Any consistent weak positive or doubtful but fluctuating reaction, sometimes seen with strong positives.
2. Any serological variations and disagreement of results, when the tests are carried out by other laboratories.
3. Negative Wassermann and positive flocculation reactions.
4. Positive tests tending to become negative within a three-months' period on weekly repetition.

The following safeguards are suggested.

1. A very careful enquiry should be made respecting any recent illness or any other condition mentioned liable to cause false positive reactions.
2. A positive result should not be regarded as an emergency: before any diagnosis is made, except possibly in pregnancy, there should be a probationary period for at least three months before commencing treatment.

3. A careful clinical examination, including radiography of the cardiovascular system, and cerebrospinal fluid tests, should be made.

4. There should be careful investigation to exclude the possibility of congenital syphilis, including the examination of infants.

5. There should be consultation with other venereal disease specialists, including the pathologist, before making a final decision.

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## DISCUSSION ON THE PRECEDING PAPER

DR. G. L. M. McELLIGOTT (the President) said he was interested to hear that the false positive Kahn reactions might possibly be caused by temperature, because this might explain certain discrepancies noticed between Kahn and Wassermann reactions during the recent cold spell. He was interested in what had been said about atypical pneumonia and had himself observed a case in which an x-ray opacity in the lung gradually vanished with the decline to negative of the false positive Wassermann.

Much had been said about false positives; he would also like to hear the speaker's views on false negatives. In many cases presented for an opinion the Wassermann was repeatedly negative, contrary to clinical findings.

DR. I. N. ORPWOOD PRICE said that when he thought of all the diseases which were said to be the cause of false positive Wassermann reactions he wondered why they bothered to do such a test. Most of this evidence came from abroad. Not very much had been produced in this country to show that false positive reactions were obtained from the diseases enumerated, and he felt that the many false positives reported must be due to faults in technique, not only in the Wassermann but also in the Kahn test. There was, moreover, little doubt that the Kahn antigen produced in America fifteen years ago was a better product than that produced now. He wondered whether commercial firms manufactured their Kahn antigen by mass production methods, which yielded an antigen more liable to give false positive reactions. In his own experience a true biological false positive was uncommon. He wondered what all this evidence really meant. He worked with clinicians who were keen, and who told him promptly if a mistake had been made. He was grateful for this co-operation; but if these false positives were so common he would have been told about them and would have had to do something. He had not studied a series of particular diseases; in fact he could think of only three types of cases which might have any bearing on the matter. First, mononucleosis; and in this disease he knew of only one patient who gave a false positive reaction. This remained positive for about a fortnight, during which five different specimens of blood were taken; each test gave a very weak positive result except the last, which was negative. Secondly, he estimated that he had examined 10,000 routine specimens from pregnant patients. He had expected 3 per cent. to be

positive. In fact, up to the present, the rate had been about 0.05 per cent. Thirdly, he had thought that high fever might give a false positive reaction, and when Mr. King was at Westbury he supplied Dr. Price with specimens of blood from patients who were undergoing high fever treatment. The specimens were taken at the height of the fever, and of 80 tests all were negative except one, an American negro suffering from syphilis. He could not help thinking that a lot of nonsense was talked about biological false positives. He would not say they did not occur, but that if they did the numbers would appear to be very small.

COL. L. W. HARRISON wished to endorse what Dr. Price had said about the importance of technique. He believed that many false positives were the result of bad technique. A number of tests called "Harrison-Wyler," carried out in this country, had given very different results with the same sera from those obtained in the Venereal Diseases Reference Laboratory, where the H-W method was the standard. Some people who said they practised that method (and he was sure this applied to the Kahn and other well-known methods) did not practise it in all its details. He would like to have sent many of the serologists whose test methods had been compared with the standard used in the Reference Laboratory to school with Dr. Wyler, who "could distinguish and divide a hair twixt north and south-west side." Dr. Wyler was unhappy if anyone walked into his laboratory smoking a cigarette, because he feared the anti-complementary effect of a flake of tobacco ash falling into one of his tubes; many serologists might copy with profit his care of glassware and of his complement sera between titration and use in the test-proper.

He thought that statements made by many authorities that pregnancy did not tend to cause false positives was based on slender evidence. Good evidence that it did so could be found in the Report of the first Serum Conference at Copenhagen, in which there are reports of tests carried out in separate laboratories between the date of the Paris Conference and that held in Copenhagen. Reports of the State Serum Institute and of the Warsaw Institute recorded many positives in pregnancy under the heading of patients without clinical evidence or history of syphilis. His own experience in the many comparisons of serum test methods carried out by the Ministry of Health's Venereal Disease Reference Laboratory between the two wars was that a shaky